

REMARKS

The Office Action of July 24, 2001 has been reviewed and its contents carefully noted. Reconsideration of this case, as amended, is earnestly requested. Claims 1-22 and 24-26 are pending in this case, claim 23 being cancelled, claims 1, 5, 6, 7 12-14, 16 and 20-21 being amended, and claims 24-26 being added by this response. The amendment of claims 1, 5, 6, 7 12-14, 16 and 20-21 is supported generally throughout the application as originally filed and, more particularly, at page 4, lines 12-16, and Figure 1. New claims 24-26 are supported throughout the application and, more particularly, at page 11, lines 3-8. No new matter has been added.

Objections to the Specification

The specification was objected to because the figure legend to Fig. 3B states that the figure shows the sequence of a 92 kb BAC (T5I7), whereas the figure does not show 92,000 bases of sequence (nor does it show any sequence).

The specification is amended to overcome the objection. More particularly, the figure legend to Fig. 3B is amended to clarify that the figure shows a graphic map of a 92 kb BAC (T5I7). It is respectfully submitted that the objection is thus overcome. Reconsideration and withdrawal of the objection are respectfully requested.

The specification was objected to because the Examiner asserts that, although all references to Figure 5 were deleted from the specification by Applicant's Preliminary Amendment, Figure 5 itself is still present in the specification, and must either be cancelled (along with any other mention of Figure 5 in the text, if any exist) or a new figure legend and the sequences presented in Figure 5 must be submitted in computer readable form.

Figure 5 was previously cancelled. See the Applicant's Preliminary Amendment, filed on June 18, 2001, at page 2, lines 11-12. If such amendment was not entered, for whatever reasons, then Figure 5 is hereby cancelled; please delete Figure 5 in its entirety. It is respectfully submitted that the objection is thus overcome. Reconsideration and withdrawal of the objection are respectfully requested.

The specification was objected to because it contains an embedded hyperlink and/or other form of browser-executable code.

The specification is amended to overcome the objection. Examples of a hyperlink or a browser-executable code are a URL placed between these symbols "<>" and http:// followed by a URL address. See MPEP § 608.01. The URL at page 10, line 23 of the present application is amended to delete "http://" therefrom, such that, as amended, it no longer constitutes an embedded hyperlink and/or other form of browser-executable code, as defined by MPEP § 608.01. It is respectfully submitted that the objection is thus overcome. Reconsideration and withdrawal of the objection are respectfully requested.

The specification was objected to because all instances of sequence in the text (e.g., page 12, paragraph 1) must be accompanied by the appropriate SEQ ID NOs. See MPEP § 2422.03 and 37 CFR 1.821(d).

The specification is amended to overcome the objection. More particularly, the specification is amended such that all instances of sequence in the text are identified by the appropriate SEQ ID NO, in accordance with MPEP § 2422.03 and 37 CFR 1.821(d). It is respectfully submitted that the objection is thus overcome. Reconsideration and withdrawal of the objection are respectfully requested.

Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 1-8, 12-14 and 16-23 were rejected under 35 U.S.C. § 112, second paragraph, as being indefinite.

The Examiner maintains that claim 1 is indefinite in its recitation of "protein involved in Vitamin C biosynthesis," as the nature of that involvement is not clear.

Claim 1 is amended to overcome the rejection. More particularly, claim 1 is amended to recite expressly an enzyme selected from a group consisting of five enzymes in the plant biosynthetic pathway for Vitamin C. It is respectfully submitted that the rejection for indefiniteness is thus overcome. Reconsideration and withdrawal of the indefiniteness rejection of claim 1 are respectfully requested.

The Examiner maintains that claim 16 is indefinite in its recitation of "enzyme crucial to Vitamin C biosynthesis," as it is not clear what level of involvement in vitamin C biosynthesis is rated "crucial".

Claim 16 is amended to overcome the rejection. More particularly, claim 16 is amended to recite expressly an enzyme selected from a group consisting of five enzymes in the plant

biosynthetic pathway for Vitamin C. It is respectfully submitted that the rejection for indefiniteness is thus overcome. Reconsideration and withdrawal of the indefiniteness rejection of claim 16 are respectfully requested.

The Examiner maintains that claims 5 and 12 are indefinite in the recitation of "is capable of overexpressing".

Claims 5 and 12 are amended to overcome the rejection. More particularly, claims 5 and 12 are amended to recite "expresses". It is respectfully submitted that the rejection for indefiniteness is thus overcome. Reconsideration and withdrawal of the indefiniteness rejection of claims 5 and 12 are respectfully requested.

The Examiner maintains that claims 6 and 13 are indefinite in the recitation of "is capable of producing".

Claims 6 and 13 are amended to overcome the rejection. More particularly, claims 6 and 13 are amended to recite expressly that the claimed plant produces increased levels of Vitamin C, relative to a progenitor plant from which said genetically engineered plant is derived, as helpfully suggested by the Examiner. It is respectfully submitted that the rejection for indefiniteness is thus overcome. Reconsideration and withdrawal of the indefiniteness rejection of claims 6 and 13 are respectfully requested.

The Examiner maintains that the terms "overexpressing" in claims 5 and 12 and "overexpression" in claim 16 are relative terms that render the claims indefinite.

Claims 5, 12 and 16 are amended to overcome the rejection. More particularly, claims 5 and 12 are amended to recite "expresses", and claim 16 is amended to recite "engineered to express". It is respectfully submitted that the rejection for indefiniteness is thus overcome. Reconsideration and withdrawal of the indefiniteness rejection of claims 5, 12 and 16 are respectfully requested.

The Examiner maintains that the term "increased" in claims 6, 13 and 20 and "increasing" in claim 16 are relative terms that renders the claims indefinite.

Claims 6, 13, 16 and 20 are amended to overcome the rejection. More particularly, claims 6, 13 and 20 are amended to recite expressly that the claimed plant produces increased levels of Vitamin C, relative to a progenitor plant from which said genetically engineered plant is derived, as helpfully suggested by the Examiner, and claim 16 is amended to recite "engineered

to express". It is respectfully submitted that the rejection for indefiniteness is thus overcome. Reconsideration and withdrawal of the indefiniteness rejection of claims 6, 13, 16 and 20 are respectfully requested.

The Examiner maintains that claims 7, 14 and 21 are not written in proper Markush format.

Claims 7, 14 and 21 are amended, as helpfully suggested by the Examiner. It is respectfully submitted that the rejection for indefiniteness is thus overcome. Reconsideration and withdrawal of the indefiniteness rejection of claims 7, 14 and 21 are respectfully requested.

The Examiner maintains that claim 20 is indefinite in its recitation of "plant ... comprises increased antioxidation capacity".

Claim 20 is amended to overcome the rejection. More particularly, claim 20 is amended to recite that the claimed plant has increased antioxidation capacity, relative to a progenitor plant from which said genetically engineered plant is derived. It is respectfully submitted that the rejection for indefiniteness is thus overcome. Reconsideration and withdrawal of the indefiniteness rejection of claim 20 are respectfully requested.

The Examiner maintains that claim 23 is indefinite in its recitation of "a form of GDP-mannose pyrophosphorylase," as it is unclear what that form is.

Claim 23 is cancelled. Reconsideration and withdrawal of the indefiniteness rejection of claim 23 are respectfully requested.

Applicant respectfully submits that these amendments have fully addressed the Examiner's rejections, that there are no other ambiguities in the claims, and that the claims are now in condition for allowance. Reconsideration and withdrawal of the rejection of claims 1-8, 12-14 and 16-23 as being indefinite are respectfully requested.

Rejections under 35 U.S.C. § 112, First Paragraph

Claim 23 was rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement.

Claim 23 is cancelled. Reconsideration and withdrawal of the rejection of claim 23 for lack of enablement are respectfully requested.

Claims 1-22 also were rejected under 35 U.S.C. § 112, first paragraph, as lacking enablement.

Applicant respectfully disagrees with the rejection, and believes that the claims, as amended, are enabled by the specification. The test for enablement is whether one reasonably skilled in the art would be able to practice the claimed invention without undue experimentation. *See e.g., Utter v Hiraga*, 845 F.2d 993, 6 USPQ2d 1709 (Fed. Cir. 1988). Thus, the specification need not (and preferably does not) disclose that which is well known in the art. *In re Buchner*, 929 F.2d 660, 661, 18 USPQ2d 1331, 1332 (Fed. Cir. 1991); *In re Myers*, 410 F.2d 420, 161 USPQ 668 (CCPA 1969); *Lindemann Maschinesfabrik GMBH v. American Hoist and Derrick Co.*, 221 USPQ 481 (Fed. Cir. 1984). It is further noted that satisfaction of the enablement requirement is not precluded by the necessity of some experimentation, such as routine experimentation. The key word here is "undue" not "experimentation". *In re Angstadt*, 190 USPQ 214 (CCPA 1976). Indeed, a considerable amount of experimentation is permissible if it is merely routine, or if the specification provides a reasonable amount of guidance to the direction in which the experimentation should proceed. *In re Jackson*, 217 USPQ 804 (Bd. App. 1982).

The Examiner maintains that the claims are broadly drawn to a method of increasing the endogenous level of vitamin C in a plant by overexpression by any method of any enzyme crucial to vitamin C biosynthesis, and plants thereby obtained.

Applicant's independent claim 1, as amended, is directed to a genetically engineered plant, comprising a recombinant nucleic acid that encodes an enzyme in a plant Vitamin C biosynthesis pathway, wherein said enzyme is selected from a group of five enzymes. As such, claim 1 (and dependent claims 2-8) is not drawn to a method, and does not recite the limitation of overexpression. Further, claim 1, as amended, is expressly limited to a group of only five enzymes. Similarly, Applicant's independent claim 9 is directed to a genetically engineered plant, comprising a recombinant nucleic acid that encodes GDP-mannose pyrophosphorylase. As such, claim 9 (and dependent claims 10-15) does not recite the limitation of overexpression. Further, claim 9 is expressly limited to a single enzyme. Independent claim 16, as amended, is directed to a method of increasing the level of Vitamin C in a plant comprising the step of engineering said plant to express an enzyme in a plant Vitamin C biosynthesis pathway, wherein said enzyme is selected from a group of five enzymes. As such, claim 16 (and dependent claims 17-22) is directed to a method, but it does not recite the limitation of overexpression. Further, claim 16, as amended, is expressly limited to a group of only five enzymes.

The Examiner asserts that the instant specification, while discussing expression of an *Arabidopsis* gene encoding GMPase in vtcl mutants of *Arabidopsis*, fails to provide guidance for successful overexpression of that gene in wild-type plants, and that overexpression of a gene in plants is unpredictable. The Examiner thus concludes that, as Applicant's gene encoding GMPase was not expressed in wild-type plants, the unpredictability associated with overexpression of genes in plants has not been overcome.

The Examiner's attention is drawn to the fact that Applicant's claims, as amended, do not encompass expression or overexpression of any gene in "wild-type plants". Rather, the claims are directed to genetically engineered plants, not wild-type plants. Therefore, no enablement regarding expression or overexpression of a gene in wild-type plants is required. Further, "overexpression" is not a feature of any of the claims, as amended. Moreover, it is respectfully submitted that the Examiner's assertion that the unpredictability associated with overexpression of genes in plants has not been overcome is mistaken, in that Applicant's gene encoding GMPase was transformed into plants, thereby generating genetically engineered plants having increased levels of Vitamin C, relative to the progenitor plants. See Applicant's specification at page 13, line 24, through page 17, line 3. Thus, clearly, Applicant's GMPase can be expressed in plants, and clearly such plants have increased levels of Vitamin C, thus overcoming any alleged unpredictability. Although in the past, in some cases expression of foreign genes in plants has been unpredictable, the current state of the art is such that, using routine methods that are well known in the art, one of ordinary skill in the art can predictably transfer into and express virtually any gene (including bacterial genes and other genes of non-plant origin) in plants (including both monocots and dicots). Indeed, the Examiner appears to have admitted such in applying the rejections under sections 102 and 103.

The Examiner asserts further that: 1) the only gene encoding GMPase taught in the instant specification is from *Arabidopsis*, 2) the instant specification fails to teach any other gene encoding any other enzyme involved in vitamin C biosynthesis, 3) it also fails to teach or suggest any method of overexpressing any enzyme other than by transformation of a gene into a plant, and 4) fails to provide guidance for the sequence of the gene encoding GMPase. Thus, the Examiner concludes that the invention appears to employ novel plasmid encoding GMPase contained in microorganisms, and that a deposit is required for enablement purposes.

It is respectfully submitted that genes encoding GMPase (including that of *Arabidopsis* and other species), as well as the other enzymes in the Vitamin C pathway, are well known in the art and, as such, are not required to be disclosed in Applicant's specification. However, Applicant's Figure 1 and the specification at page 4, lines 12-16, disclose the enzymes in the

Vitamin C pathway. Further, Applicant's specification provides ample guidance for the sequence of the gene encoding GMPase at page 11, lines 5-8, wherein the GenBank accession number is provided. Thus, one of ordinary skill in the art would know precisely the sequence of the GMP-mannose pyrophosphorylase, as it is well known in the art, and ample guidance thereto also is disclosed in Applicant's specification. Finally, the claims, as amended, do not encompass any method for expressing a recombinant gene in a plant, other than that of transformation of a gene into a plant. Therefore, no guidance regarding any other methods is required.

In view of the above, the examiner is requested to note that the specification contains sufficient matter to support the genetically engineered plants and the method of generating such plants, as presently claimed. In particular, the specification discloses the appropriate elements of the claimed plants together with the appropriate techniques that would effectively produce such plants. Indeed, the specification provides one skilled in the art with the detailed steps deemed necessary to duplicate the claimed method and genetically engineered plants, including methods for generating mutations in other genes encoding enzymes in the Vitamin C biosynthetic pathway, methods for identifying and isolating genes, methods for transforming plants to express genes, and methods for screening and selecting plants having increased levels of Vitamin C. Thus, it is respectfully submitted that the disclosure complies with section 112 fully, in that the description of the various elements of the genetically engineered plants, together with the steps relating to the novel process for making such plants, provides a full, clear and concise instruction to any person reasonably skilled in the art, thus enabling him to make and use the invention without undue experimentation.

In view of the foregoing amendments and remarks, Applicant respectfully submits that these amendments have fully addressed the Examiner's rejections, and that the claims are now in condition for allowance. Reconsideration and withdrawal of the rejection of claims 1-23 as lacking enablement are respectfully requested.

Claims 1-23 were rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter that was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. More particularly, the Examiner maintains that the specification does not describe which enzymes are crucial for vitamin C biosynthesis, their enzymatic activity, or the sequence of any gene encoding any enzyme involved in vitamin C biosynthesis, including that of any gene encoding GMPase, and does not demonstrate the isolation of GMPase genes from plants other than *Arabidopsis*. Therefore, the Examiner concludes that, given the lack of written description in the specification with regard to the

structural and physical characteristics of the claimed plants and methods, and given the high level of unpredictability in this art, one skilled in the art would not have been in possession of the genus claimed at the time this application was filed.

It is respectfully submitted that genes encoding GMPase (including that of *Arabidopsis* and other species), as well as the other enzymes in the Vitamin C pathway, are well known in the art and, as such, are not required to be disclosed in Applicant's specification. However, Applicant's Figure 1 and the specification at page 4, lines 12-16, disclose the enzymes in the Vitamin C pathway. Further, Applicant's specification provides ample guidance for the sequence of the gene encoding GMPase at page 11, lines 5-8, wherein the GenBank accession number is provided. Thus, one of ordinary skill in the art would know that Applicant was in possession of the sequence of the GMP-mannose pyrophosphorylase, as it is well known in the art, and ample guidance thereto also is disclosed in Applicant's specification.

Moreover, it is respectfully submitted that the Examiner's assertion that the unpredictability associated with overexpression of genes in plants has not been overcome is mistaken, in that Applicant's gene encoding GMPase was transformed into plants, thereby generating genetically engineered plants having increased levels of Vitamin C, relative to the progenitor plants. See Applicant's specification at page 13, line 24, through page 17, line 3. Thus, clearly, Applicant's GMPase can be expressed in plants, and clearly such plants have increased levels of Vitamin C, thus overcoming any alleged unpredictability.

In view of the foregoing amendments and remarks, Applicant respectfully submits that these amendments have fully addressed the Examiner's rejections, and that the claims are now in condition for allowance. Reconsideration and withdrawal of the rejection of claims 1-23 as lacking a sufficient written description are respectfully requested.

Rejection under 35 U.S.C. § 101

Claim 23 was rejected as claiming unpatentable subject matter under 35 U.S.C. § 101.

Claim 23 is cancelled. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejections under 35 U.S.C. § 102

Claims 1-2, 5-8, 16-18 and 20-22 were rejected under 35 U.S.C. § 102(e) as being clearly anticipated by Trulson *et al.* (U.S. Patent No. 6,143,562, filed April, 1995). The Examiner

maintains that Trulson *et al.* teaches tomato, melon, squash and maize plants transformed with a gene encoding phosphomannose isomerase, an enzyme in the vitamin C biosynthetic pathway. The Examiner further maintains that the claimed increased stress resistance would have been an inherent property of these plants, as would increased vitamin C levels.

Independent claims 1 and 16 are amended to overcome the rejection. More particularly, claims 1 and 16 are amended to recite expressly an enzyme selected from a group consisting of five enzymes in the plant biosynthetic pathway for Vitamin C, clearly excluding phosphomannose isomerase. It is respectfully submitted that the rejection for anticipation is thus overcome. Reconsideration and withdrawal of the anticipation rejection of independent claims 1 and 16 are respectfully requested.

Dependent claims 2, 5-8, 17-18 and 20-22, being dependent upon and further limiting independent claims 1 and 16, should be allowable for the same reason, as well as for the additional recitations they contain. Reconsideration and withdrawal of the anticipation rejection of independent claims 1-2, 5-8, 16-18 and 20-22 are respectfully requested.

Claims 1-3, 5-8, 16, and 18-22 were rejected under 35 U.S.C. § 102(a) as being clearly anticipated by Bauw *et al.* (WO 98/50558). The Examiner maintains that Bauw *et al.* teaches *Arabidopsis* and tobacco plants transformed with a gene encoding L-galactono-y-lactone dehydrogenase, an enzyme involved in vitamin C biosynthesis. The Examiner further maintains that the resulting plants overproduce vitamin C, and would be stress resistant.

Independent claims 1 and 16 are amended to overcome the rejection. More particularly, claims 1 and 16 are amended to recite expressly an enzyme selected from a group consisting of five enzymes in the plant biosynthetic pathway for Vitamin C, clearly excluding L-galactono-y-lactone dehydrogenase. It is respectfully submitted that the rejection for anticipation is thus overcome. Reconsideration and withdrawal of the anticipation rejection of independent claims 1 and 16 are respectfully requested.

Dependent claims 2-3, 5-8 and 18-22, being dependent upon and further limiting independent claims 1 and 16, should be allowable for the same reason, as well as for the additional recitations they contain. Reconsideration and withdrawal of the anticipation rejection of independent claims 1-3, 5-8, 16, and 18-22 are respectfully requested.

Rejection under 35 U.S.C. § 103

Claims 3 and 19 were rejected under 35 U.S.C. § 103(a) as being unpatentable over Trulson *et al.* in view of Schmidt *et al.* (1988, Plant Cell Rep. 7:583-586). The Examiner maintains that Trulson *et al.* discloses various dicots transformed with a gene encoding phosphomannose isomerase, an enzyme in the vitamin C biosynthetic pathway, and that these plants would have increased stress resistance and vitamin C levels. Schmidt *et al.* teaches transformation of *Arabidopsis*. Therefore, the Examiner concludes that, at the time the invention was made, it would have been obvious to one of ordinary skill in the art to transform dicots with a gene encoding an enzyme in the vitamin C biosynthetic pathway, as taught by Trulson *et al.*, and to modify that to transform *Arabidopsis* with that gene, as described in Schmidt *et al.* The Examiner also concludes that "One of ordinary skill in the art would have been motivated to do so.", however, no support for that assertion is provided.

Applicant respectfully disagrees with the rejection, and believes the claims, as amended, are patentable over Trulson *et al.* and Schmidt *et al.*, individually and in combination, for the reasons given above in respect to the section 102 rejection of claims 1 and 16, from which claims 3 and 19 depend. The argument above as to the novelty of claims 1 and 16 is repeated here by reference. The combination of Trulson *et al.* and Schmidt *et al.* would not teach or suggest a genetically engineered plant that expresses any of the five enzymes claimed by Applicant, or a method for making such a plant. Thus, it is respectfully submitted that the rejection is overcome. Reconsideration and withdrawal of the obviousness rejection of claims 3 and 19 are respectfully requested.

Conclusion

Applicant believes the claims, as amended, are patentable over the prior art, and that this case is now in condition for allowance of all claims therein. Such action is thus respectfully requested. If the Examiner disagrees, or believes for any other reason that direct contact with Applicants' attorney would advance the prosecution of the case to finality, he is invited to telephone the undersigned at the number given below.

"Recognizing that Internet communications are not secured, I hereby authorize the PTO to communicate with me concerning any subject matter of this application by electronic mail. I understand that a copy of these communications will be made of record in the application file."

Respectfully Submitted:

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10/24/01
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Marked-up Copy of Amended Claims in Compliance with C.F.R. § 1.121(c):

1. (Amended) A genetically engineered plant, or portion thereof, comprising a recombinant nucleic acid [sequence] that encodes [a protein involved] an enzyme in a plant Vitamin C biosynthesis pathway, wherein said enzyme is selected from the group consisting of phosphoglucose isomerase, phosphomannomutase, GDP-mannose pyrophosphorylase, GDP-mannose epimerase and galactonolactone dehydrogenase.
5. (Amended) The genetically engineered plant of claim 1 wherein said genetically engineered plant, or portion thereof, [is capable of overexpressing] expresses said recombinant nucleic acid.
6. (Amended) The genetically engineered plant of claim 1 wherein said genetically engineered plant, or portion thereof, [is capable of producing] produces increased levels of Vitamin C, relative to a progenitor plant from which said genetically engineered plant is derived.
7. (Amended) The genetically engineered plant of claim 1 wherein said genetically engineered plant, or portion thereof, has increased resistance to environmental stress compared to a plant of the same species without said recombinant nucleic acid wherein said environmental stress is selected from the group consisting of[: a)] drought[:], [b)] cold[:], [c)] UV radiation[:], [d)] air pollution[:], [e)] salts[:], [f)] heavy metals[:] and [g)] reactive oxygen species.
12. (Amended) The genetically engineered plant of claim 9 wherein said genetically engineered plant, or portion thereof, [is capable of overexpressing] expresses said recombinant nucleic acid.
13. (Amended) The genetically engineered plant of claim 9 wherein said genetically engineered plant, or portion thereof, [is capable of producing] produces increased levels of Vitamin C, relative to a progenitor plant from which said genetically engineered plant is derived.
14. (Amended) The genetically engineered plant of claim 9 wherein said genetically engineered plant, or portion thereof, has increased resistance to environmental stress compared to a plant of the same species without said recombinant nucleic acid wherein said environmental stress is selected from the group consisting of[: a)] drought[:], [b)] cold[:], [c)] UV radiation[:], [d)] air pollution[:], [e)] salts[:], [f)] heavy metals[:] and [g)] reactive oxygen species.

16. (Amended) A method of increasing the [endogenous] level of Vitamin C produced in a plant, or portion thereof, comprising [overexpression of an enzyme crucial to Vitamin C biosynthesis] the step of:
- engineering said plant, or portion thereof, to express a recombinant nucleic acid that encodes an enzyme in a plant Vitamin C biosynthesis pathway, wherein said enzyme is selected from the group consisting of phosphoglucose isomerase, phosphomannomutase, GDP-mannose pyrophosphorylase, GDP-mannose epimerase and galactonolactone dehydrogenase.
20. (Amended) The method of claim 16 wherein said plant, or portion thereof, comprises increased antioxidation capacity, relative to a progenitor plant from which said genetically engineered plant is derived.
21. (Amended) The method of claim 16 wherein said plant, or portion thereof, has increased resistance to environmental stress compared to a plant of the same species without said recombinant nucleic acid wherein said environmental stress is selected from the group consisting of: a) drought[;], b) cold[;], c) UV radiation[;], d) air pollution[;], e) salts[;], f) heavy metals[;] and g) reactive oxygen species.

Marked-up Copy of Amendments to Specification in Compliance with C.F.R. § 1.121(b):

Fig. 3B shows [the sequence] a graphic map of a 92 kb BAC (T5I7) within the contig of Fig. 3A.

Using a mapping population of >400 F3 families derived from a cross between *vtc1-1* and the wildtype *Ler* ecotype, *VTCL* was fine-mapped to a position on chromosome 2 to one side of two molecular markers; 0.9 cM from marker m429 and 1.2 cM from marker nga168 (as shown in Figure 3A). Using microsatellite marker 178, which is >1 cM centromeric proximal to nga168, it was determined that *VTCL* is centromere distal to nga168 and m429. All seven *vtc1/vtc1* mapping lines that were recombinant between nga168 and *VTCL* were also recombinant for marker 178 (including two between m429 and nga168), indicating that the relative order of these loci is as shown. This map is inconsistent with public domain recombinant inbred results, presumably because of the limited resolution of the recombinant inbred map: m429 is reported as being centromere proximal to nga168 (http://nasc.nott.ac.uk/new_ri_map.html). Our mapping data place *VTCL* within a 2 Mb region on Chr 2 that spans m429 to just beyond marker m336, which is currently being sequenced by the Institute for Genomic Research (TIGR). The sequence of a 92 kb BAC (T5I7) within that contig (Figure 3B) was annotated by TIGR and the open reading frame T5I7.7 was identified as a putative mannose-1-phosphate guanylyltransferase (www.tigr.org/docs/tigr-scripts/bac_scripts/bac_display.spl?bac_name=T5I7). An alias for this enzyme is GDP-mannose pyrophosphorylase, which catalyzes step 4 in the proposed AsA biosynthetic pathway shown in Fig. 1. In this reaction, mannose-1-P is converted to GDP-mannose, with the consumption of GTP and the release of inorganic pyrophosphate (PPi).

The mutant alleles *vtc1-1* and *vtc1-2* were sequenced from PCR-amplification products of genomic DNAs. For each mutant allele, an ~1.4 kb BglII fragment containing the majority of the coding region was sequenced using the primers, 5' TGGTAAATACGCACTCAAT 3' (SEQ ID NO: 1, named 5'-GMP) and 5' AAAACAGCAAACGACCCTAACAA 3' (SEQ ID NO: 2, named 3'-GMP). To confirm the public domain sequence of BAC T5I7 that included the base mutated in the *vtc1* alleles, both strands of a portion of a Col-0 wildtype *VTCL* ClaI genomic clone (described below) were sequenced. The sequence of *VTCL*, *vtc1-1*, and *vtc1-2* that included exon 1 and intron 1 was obtained directly from genomic DNA amplified with 5'-GMP and 5' CATTCTTGTTGGAGGCTTCGG 3' (SEQ ID NO: 3). The sequence downstream of the

BglI fragment for *vtcI-1* and *vtcI-2* was obtained from genomic DNA amplified with the 5' GAATAAGCATCAATCAAAACGC 3' (SEQ ID NO: 4) and 5' GCTAAGACCGACTTCAATCG 3' (SEQ ID NO: 5). More than one independent PCR product was sequenced to confirm the veracity of the data.